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NOTES

Evaluation of the Biolog Automated Microbial Identification System

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Biolog's identification system was used to identify 39 American Type Culture Collection reference taxa and 45 gram-negative isolates from water samples. Of the reference strains, 98% were identified to genus level and 76% to species level within 4 to 24 h. Identification of some authentic strains of *Enterobacter*, *Klebsiella*, and *Serratia* was unreliable. A total of 93% of the water isolates were identified.

Although rapid automated identification systems for identifying bacteria from clinical specimens exist, few systems that can identify environmental isolates quickly and reliably are available (3, 4, 8, 27). Traditional biochemical tests and commercial test kits used to identify nonenteric organisms are labor-intensive and slow (9, 10, 13, 14, 16, 19, 21–24, 26, 28, 29, 31). An automated ground-based system is needed for identifying archived environmental isolates obtained from the space shuttle and eventually from the U.S. space station *Freedom* (1, 20). Identification of isolates from the station's recycled water supply is of particular concern.

Biolog Inc., (Hayward, Calif.) recently introduced an automated system designed to identify 434 species or groups of aerobic gram-negative bacteria within 4 to 24 h. Their data base includes environmental taxa that are not included in the data bases of other commercial systems (3, 4). Because the Biolog system has been available for a relatively short time, few published assessments of its accuracy and reliability are available (6, 18). To assess the system's potential to identify gram-negative aerobic rods, we tested 41 American Type Culture Collection (ATCC) organisms representing 39 taxa and 45 unknown isolates recovered from a prototype water-recycling system proposed for use on the U.S. space station *Freedom*.

Biolog's identification system, consisting of a Microstation computer, turbidimeter (optical density at 590 nm), MicroLog software (MicroLog 2N, Release 2), microplate reader, and gram-negative microplates, was purchased from Biolog Inc. For comparative purposes, the Vitek system, turbidimeter, and gram-negative identification cards were purchased from Vitek Systems, Inc. (Hazelwood, Mo.). Petri plates (160-mm diameter) prepared with Trypticase soy agar, blood agar (Trypticase soy agar plus 5% sheep erythrocytes), plate count agar, or Emmon's Sabouraud dextrose agar were purchased from BBL Microbiology Systems (Cockeysville, Md.). Sterile saline (0.45 and 0.85%) was purchased from Baxter Healthcare Corp. (Deerfield, Ill.). Cards, microplates, and agar plates were stored at 4°C and then equilibrated at room temperature for 30 min (cards and microplates) or overnight (agar plates) before use.

Authentic isolates representing 39 taxa were obtained from the ATCC to serve as reference isolates (see Table 1). The ATCC isolates were rehydrated according to the method recommended by the ATCC and subcultured onto Trypticase soy agar or blood agar plates, which were then incubated at 30 or 35°C for 24 to 48 h. Isolated colonies were restreaked to ensure purity and incubated overnight. Inocula were prepared according to the manufacturers' instructions (5, 30). The isolates were then tested (in triplicate) by using both the Biolog and Vitek systems.

Forty-five isolates consisting of eight morphologically distinct colony types were recovered from a prototype water-recycling system (Marshall Space Flight Center, Huntsville, Ala.) being tested for use on the space station *Freedom* (1). Water samples collected from the system's distribution lines, hygiene waste storage tank outlets, and humidity condensate storage tank outlets were shipped overnight to the Johnson Space Center (Houston, Tex.) in polyethylene bottles at 4°C. Sodium thiosulfate (100 mg/liter) was added at the time of collection to inactivate trace levels of iodine (12). Organisms were recovered from water samples by standard membrane filtration or spread plate techniques on plate count agar (12); each isolate was subcultured on Trypticase soy agar or blood agar (Sabouraud dextrose agar was used if a *Methylobacterium* sp. was suspected) and incubated for 24 to 48 h at 30°C.

Biolog identifications were compared with the results obtained with the Vitek system (see Tables 1 and 2). If neither system could identify a water isolate, or if the identification of an isolate was not confirmed by the second automated system, Gram stain reaction and colony morphology characteristics were recorded, catalase, oxidase, and indole spot tests were performed, and the isolate was archived (stored in 20% glycerol and 80% Trypticase soy broth at −70°C) for future analysis.

Interpretation of results. Biolog identifications were accepted as correct if the similarity index of the genus and species name was 0.750 or greater at 4 h or 0.500 or greater at 24 h. The Vitek identifications were accepted if the assigned identity met or exceeded 92% probability. In addition to meeting these manufacturers' criteria, sample identities were accepted as correct if the sample organism was listed in the system's data base and if the assigned identity either matched the genus and species of the reference ATCC strain or could be verified by the second automated system. The identities of the unknown water isolates are discussed below.

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TABLE 1. Identification of ATCC strains

Organism	Biolog			Vitek result (no. correctly identified/no. of replicates)
	No. correctly identified/no. of replicates at:		Incorrect identification(s) at 24h[a]	
	4 h	24 h		
Acinetobacter baumannii (ATCC 19606)[b]	0/3	4/6	*Acinetobacter calcoaceticus* (2)	6/6
Aeromonas hydrophila (ATCC 49140)	3/3	3/3		3/3
Alcaligenes faecalis (ATCC 8750)	3/3	3/3		3/3
Bordetella bronchiseptica (ATCC 10580)[b]	6/6	0/6	*Philomiragia bacterium* (6)	6/6
Citrobacter diversus (ATCC 27156)	0/3	3/3		3/3
Citrobacter freundii (ATCC 8090)[b]	0/6	5/6	No ID (1)	6/6
Enterobacter agglomerans (ATCC 27155)[b,c]	3/6	6/6		3/3
Enterobacter aerogenes (ATCC 13048)[b]	0/9	6/9	No ID (3)	9/9
Enterobacter cloacae (ATCC 13047)[c]	0/3	0/3	*Klebsiella taylorae*, K. terrigena (2); no ID (1)	3/3
Escherichia coli (ATCC 25922)[b]	3/3	5/6	No ID (1)	6/6
Escherichia coli (ATCC 35218)	3/3	0/3	*K. terrigena* (3)	3/3
Hafnia alvei (ATCC 29926)[b]	0/6	5/6	No ID (1)	6/6
Flavobacterium indologenes (ATCC 29897)[b]	0/6	0/6	*Flavobacterium gleum* (6)	0/6 (*Flavobacterium* sp.)
Flavobacterium odoratum (ATCC 4651)[b]	0/6	0/6	*Weeksella virosa* (3); no ID (3)	0/6 (no ID; NFGNR[d])
Klebsiella oxytoca (ATCC 43863)[b]	4/9	1/9	*K. terrigena*, *K. pneumoniae* (7); no ID (1)	3/3
Klebsiella ozaenae (ATCC 11296)[b]	5/6	0/6	*K. terrigena* (3); no ID	6/6
Klebsiella pneumoniae (ATCC 27736)[b]	0/9	1/9	*K. terrigena* (2); no ID (6)	7/7
Klebsiella planticola (ATCC 33531)[b,c]	3/6	1/6	*K. terrigena*, *E. aerogenes*, *Klebsiella trevisanii* (5)	
Klebsiella terrigena (ATCC 33257)[b]	0/6	0/6	*K. planticola*, *K. ozaenae* (3)	
Klebsiella rhinoscleromatis (ATCC 13884)	0/3	3/3		3/3
Methylobacterium fujisawaense (ATCC 35065)[b]	0/6	0/6	*M. rhodinum* (6)	
Methylobacterium rhodinum (ATCC 14821)[b]	0/6	0/6	*Methylobacterium* sp. subgroup A (6)	
Methylobacterium organophilum (ATCC 27886)[b]	0/6	0/6	*Methylobacterium* sp. subgroup A (5); no ID (1)	
Proteus mirabilis (ATCC 7002)[b]	0/6	2/6	No ID (4)	9/9
Pasteurella multocida (ATCC 43137)	0/3	3/3		3/3
Pseudomonas aeruginosa (ATCC 27853)[b]	0/3	9/9		6/6
Pseudomonas cepacia (ATCC 35254)	0/3	3/3		3/3
Pseudomonas diminuta (ATCC 19146)	0/3	3/3		
Pseudomonas fluorescens (ATCC 13525)	1/3	3/3		3/3
Pseudomonas paucimobilis (ATCC 29837)	0/3	3/3		3/3
Pseudomonas pickettii (ATCC 49129)[c]	0/3	3/3		3/3
Pseudomonas pickettii (ATCC 27511)[b]	6/6	9/9		7/9
Pseudomonas putida (ATCC 49128)[b]	2/6	3/6	No ID (1)	3/3
Salmonella typhimurium (ATCC 14028)[b,f]	2/6	3/6	No ID (1)	
Serratia liquefaciens (ATCC 35551)[b]	6/9	1/9	No ID (2)	3/3
Serratia rubidaea (ATCC 33670)[b]	6/9	1/9	*K. terrigena* (2)	4/4
Serratia marcescens (ATCC 14756)[b]	0/6	0/6	*Serratia ficaria* (1); no ID (5)	6/6
Shewanella putrefaciens (ATCC 49138)[b]	3/6	5/6	No ID (1)	6/6
Shigella sonnei (ATCC 25931)[b]	3/3	6/6		6/6
Xanthomonas maltophilia (ATCC 49130)[b]	1/9	4/9	*Pseudomonas glycinea* (1); no ID (4)	6/6
Yersinia enterocolitica (ATCC 23715)[b]	6/6	6/6		6/6

[a] Numbers in parentheses indicate the number of cultures for which that identification was assigned.
[b] Additional sets of triplicates were selected at random for retesting.
[c] All replicates were identified correctly after 6 to 8 h of incubation by Biolog's 4-h data base reactions.
[d] NFGNR, nonfermenting gram-negative rod.
[e] Purchased as *P. cepacia* but verified by ATCC as a variant of *P. pickettii*.
[f] Included in Biolog's data base as *Salmonella* sp. subsp. 1, subgroup G.

Identification of ATCC strains. The Biolog system's identifications of the 41 authentic ATCC isolates representing 39 taxa are shown in Table 1. Because assigned identities occasionally changed as the incubation progressed, results at 4 h and at 24 h are presented separately. If a sample's similarity index was below 0.750 at 4 h, the Biolog system issued instructions to continue the incubation to 24 h. Of the 41 ATCC isolates, 40 (98%) were identified to the correct genus and 24 (59%) were identified to the correct species. Of the 41 strains, 31 (76%) could be identified to the species level with reasonable certainty, if the identity of enteric organisms assigned at 4 h was accepted. The remaining 10 species (24.4%) were either consistently misidentified or assigned no identity for >67% of the replicates tested (*Enterobacter cloacae*, two *Flavobacterium* spp., *Klebsiella pneumoniae*, *Klebsiella terrigena*, three *Methylobacterium* spp., *Proteus mirabilis*, and *Serratia marcescens*).

The 39 reference taxa (ATCC strains) tested included

TABLE 2. Identification of unknown water isolates

Location[a]	No. of isolates	Identification(s)	
		Biolog	Vitek
Hygiene waste	4	*C. freundii*	*C. freundii*
Hygiene waste	3	*E. hermannii*	*E. hermannii*
Hygiene waste	1	*Enterobacter* sp., *E. cloacae*	*E. cloacae*
Hygiene waste	2	*E. cloacae*, *K. pneumoniae*[b]	*E. cloacae*
Hygiene waste	1	*Enterobacter intermedium*	*Kluyvera* sp.
Hygiene waste	2	*E. intermedium*, *Kluyvera cryocrescens*[b]	*Kluyvera* sp.
Hygiene waste	1	*K. oxytoca*	*K. pneumoniae*
Hygiene waste	1	*K. ozaenae*, *K. oxytoca*[b]	*K. pneumoniae*
Hygiene waste	2	*K. planticola*	*K. pneumoniae*
Hygiene waste	3	*P. aeruginosa*	*P. aeruginosa*
Hygiene waste	1	*P. fluorescens*	*P. fluorescens*
Hygiene	1	*P. pickettii*	*P. pickettii*
Hygiene waste	2	*P. putida*	*P. putida*
Hygiene waste	2	*S. marcescens*	*S. marcescens*
Condensate	1	*Methylobacterium* sp. subgroup A	No ID[c]
Potable; condensate	2	*M. rhodinum*	No ID[c]
Condensate	1	*M. rhodinum* or *Methylobacterium* sp. subgroup A	No ID[c]
Condensate	1	*Moraxella atlantae*	No ID[c]
Condensate	3	No ID[c]	No ID[c]
Condensate	3	*P. paucimobilis*	*P. paucimobilis*; no ID[c]
Condensate	3	*P. pickettii*	*P. pickettii*
Condensate	2	*Pseudomonas* sp.	*Flavobacterium* sp.
Condensate	3	CDC group IV2	*Flavobacterium* sp.; NFGNR[d]; no ID[c]

[a] Hygiene waste, hygiene waste storage tank outlet; hygiene, hygiene distribution line; condensate, condensate waste storage tank; potable, potable distribution line.

[b] The isolate was identified at 4 h as the first organism and at 24 h as the second. All identifications had similarity indices above 0.750 at 4 h or 0.500 at 24 h (see text).

[c] No ID, system could not match the metabolic reactions to any in its data base.

[d] NFGNR, nonfermenting gram-negative rod.

seven species that were not part of the Vitek data base. Of these, the Vitek system named *Pseudomonas diminuta* (ATCC 19146) as an unidentified nonfermenting gram-negative rod and identified *Salmonella typhimurium* (ATCC 14028) to the genus level. *K. terrigena* (ATCC 33257) and *Klebsiella planticola* (ATCC 33531) were identified as *K. pneumoniae*. The three *Methylobacterium* species were reported as "unidentified." Overall, the Vitek system identified 88% (36 of 41) of the reference isolates to the correct genus and 78% (32 of 41) to the correct species. The system also correctly identified 94% (32 of 34) of the strains it was designed to identify (Table 1).

Identification of water isolates. Identities assigned to the 45 isolates recovered from the prototype water-recycling system are shown in Table 2. *Escherichia hermannii*, *E. cloacae*, *Citrobacter freundii*, *S. marcescens*, and five *Pseudomonas* spp. were identified by both the Biolog and the Vitek systems. Several *Methylobacterium* isolates (see below) were identified to genus level by the Biolog system but not by the Vitek system. Some *Klebsiella*, *Enterobacter*, and *Kluyvera* species identifications tended to change between 4 and 24 h; the Vitek identification of *K. planticola* as *K. pneumoniae* probably reflects the absence of *K. planticola* in the Vitek data base. In summary, the Biolog system assigned identities to 42 of the 45 water isolates (93%) and the Vitek system identified 37 (82%). The number of isolates that were assigned the same identity by both systems was approximately 68% (25 of 37). The remaining isolates were archived for future analysis.

The results of this study agree with those of Miller and Rhoden (18), who observed that accuracy of the identification of some enteric species can be improved by incubating the microplates for 6 to 8 h and using the 4-h data base reactions. Although this approach is not recommended by the manufacturer, we also found that this procedure increased the accuracy of identification for some enteric isolates (Table 1). For example, extending the incubation period for *E. cloacae* and *Enterobacter agglomerans* to 6 to 8 h resulted in consistently correct identifications, although *S. marcescens*, which grows quickly, was difficult to identify at any incubation period. In other studies, the Biolog system correctly identified 116 of 130 infrequently isolated gram-negative bacteria (76% identified to the correct species, with an additional 12% identified to the correct genus but the incorrect species) (17), correctly identified 27 of 46 isolates from human sources to the species level and 11 to the genus level (2), and correctly identified 86% of *Pseudomonas cepacia* strains isolated from the sputum of patients with cystic fibrosis (25). We have found Biolog's identification of *P. cepacia* and *Pseudomonas pickettii* isolated from various water supplies to be consistently correct (our unpublished data). Carson and Miller (7) have recommended expanding the Biolog data base to reclassify the pink-pigmented organisms. In the present study, the Biolog system identified selected pink-pigmented gram-negative rods as belonging to the genus *Methylobacterium*. Biolog's prior genus identification of three ATCC *Methylobacterium* spp. (Table 1) and the ability of these isolates to metabolize formate and other sugars support this characterization (11, 15, 32). Verification of the identification of the *Methylobacterium* isolates to species level was not attempted.

We found the Biolog system easy to use, to update, and to customize for specific needs. Its accuracy in identifying nonenteric organisms is satisfactory. However, incorrect

identifications and identifications that change with incubation time detract from the system's usefulness. Part of the difficulty in reliable identification of enteric organisms could be alleviated by testing for oxidase, catalase, indole, and motility when the microplate is inoculated. Alternatively, the data base for enteric organisms, or the recommended optical density of the inoculum for enteric species, could be modified. The data base for the identification of the *Methylobacterium* isolates to species level needs to be reassessed. The large number of organisms in Biolog's data base warrants ongoing verification of assigned identities with known authentic isolates. In summary, although the Biolog system offers great promise, its identifications should be viewed cautiously.

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